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In vitro evaluation of antidiabetic activity of methanol extracts of selected medicinal plants

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ABSTRACT:

Diabetes mellitus is a chronic disease characterised by insulin insufficiency, which results in hyperglycaemia and causes acute and chronic consequences. The goal of this study is to determine the antidiabetic activity of methanol extracts of five medicinal plants in vitro using an enzyme inhibition test for α-amylase and α-glucosidase. The study used acarbose and methanol extracts from all five plants at doses of 10, 20, 30, 40, 50, 60, 70, and 80 µg/ml. The absorbance values for -amylase and α-glucosidase enzymes were measured in a spectrophotometer at 540 nm and 546 nm, respectively. The extracts significantly inhibited the enzymes -amylase and α-glucosidase in a dose-dependent manner. When compared to other plants, *Phyllanthus madraspetensis* methanol extract had greater inhibitory effect against α-amylase and α-glucosidase enzymes. We concluded that *Phyllanthus madraspetensis* methanol extract showed better antidiabetic effect in in vitro models based on our findings. This finding calls for more research and animal model tests.

Keywords: Diabetes mellitus, alpha-glucosidase, alpha-amylase.

Introduction:

Diabetes mellitus is a broad term for a collection of diseases that affect the metabolism of biomolecules including carbs, lipids, and proteins. According to surveys conducted by the World Health Organization, there were 171 million cases of diabetes globally in 2000. By 2030, it is expected that the number would have risen to 366 million (1). Diabetes is divided into two types: insulin-dependent diabetes (type 1) and non-insulin-dependent diabetes (type 2). (type-2). Type 1 diabetes is an autoimmune illness marked by a localized inflammatory response in and around the pancreas, followed by the selective death of insulin-secreting -cells. Peripheral insulin resistance and decreased insulin secretion are hallmarks of type 2 diabetes (T2D) (2, 3). Diabetes is prevalent and has serious repercussions in countries such as India (31.7 percent), China (20.8 percent), and the United States (17.17 percent). By 2030, the rates are predicted to climb to 79.4%, 42.3 percent, and 30.3 percent, respectively (4). Diabetes mellitus is defined by hyperglycemia, which is caused by an absolute or relative insulin deficit, and is linked to long-term problems that damage the eyes, kidneys, heart, and nerves (5). There is still no adequate effective treatment or therapy to cure diabetes in modern medicine (6).

Medicinal plants and their compounds, on the other hand, that are widely prescribed for the treatment of T2D around the world have no recognized scientific basis for their efficacy. As a result, medicinal plants must be thoroughly investigated in order to determine their ability to treat both chronic and acute ailments. Twenty plants were studied in an attempt to find new sources that could be used to treat T2D, including *Abutilon indica*, *Acalypha fruticosa*, *Alangium salvifolium*, *Amarnthus virudis*, *Alternanthera sessile*, *Alternanthera tenella*. These five plants were chosen for this investigation in order to demonstrate scientifically.

However, several synthetic medications are accessible as oral hypoglycemic agents and diabetes drugs, but long-term usage of synthetic pharmaceuticals causes significant adverse effects and is extremely costly. Hypoglycemic agents derived from natural products, particularly plants, have recently gained popularity due to their fewer side effects, and these plants contain bioactive compounds known as secondary metabolites, which are not involved in plant growth but have a variety of biological activities such as antibacterial, antidiabetic, anti-inflammatory, anticancer, and so on. As a result, the current study aims to evaluate the biological potentials of methanol extracts of five medicinal plants, including -amylase and -glucosidase activity, in order to identify effective medicines for dual therapy of both AD and T2D.

Materials and Methods:

Sample preparation

The plant species were chosen based on pharmacological and cost-effectiveness, as well as health benefit claims, according to the Unani system of medicine. Twenty herbs were used, including *Abutilon indica*, *Acalypha fruticosa*, *Alangium salvifolium*, *Amarthus virudis*, *Alternanthera sessile*, *Alternanthera tennela*, *Boerhavia diffusa*, *Calophyllum inophyllum*, *Corchorus aestuans*, *Eincostemma axill.* In the College herbarium, a voucher specimen was placed. Table 1 lists the entire details of the experimental plants for each reference.

Grinding followed by fine sifting of the plant material yielded 250 gms of fine powder from plant leaves. 90 percent methanol was used to dissolve the powder. The solvent containing plant ingredients was filtered with What Man No. 1 filter paper after 15 days to remove plant debris. To separate the solvent from the plant ingredients, the filtrate was concentrated using a rotary and evaporated using a rotary evaporator (Buchi R-210) at low pressure (60RPM/Min) at 40°C gradually increased to 80°C. Crude extract is collected using chloroform and methanol once the solvent has been completely separated. The extract is now collected in a collecting vessel, freeze-dried, and kept at 20°C until needed in the experiment.

Plant extracts preparation:

The dried plant extracts were weighed and diluted in sterile distilled water to make the needed dilutions of 10, 20, 30, 40, 50, 60, 70, and 80 µg/ml. Unless they were utilized in the experiment, they were kept in the refrigerator.

α-amylase inhibition activity:

α-Amylase inhibitory activity was measured using a modified version of the Xiao et al. (2006) method based on the starch iodine test. The total assay mixture was incubated at 37°C for 10 minutes, consisting of 0.2M sodium phosphate buffer (pH 6.9 containing 6 mm sodium chloride), 10 l salivary amylase, and 50 l various plant extracts with concentrations ranging from 10-80 gml-1 (w/v). Each reaction mixture was then given 150 microliters of starch (1 percent w/v) and incubated for 15 minutes at 37°C. After that, 20 microliters of HCl (1 M) was added to stop the enzymatic reaction, and then 20 l of iodine reagent was added (5 mm I2 and 5 mm KI). Microplate reader (iMarkTM Microplate Absorbance Reader) was used to detect the color shift and record the absorbance at 620 nm. There was no plant extract in the control reaction tubes, which represented 100% enzyme activity. Appropriate extract controls lacking the enzyme were also evaluated to eliminate the absorbance produced by plant extract. The presence of starch is indicated by a dark-blue color; the lack of starch is indicated by a yellow color; and the presence of partially degraded starch in the reaction mixture is indicated by a brownish color. The starch added to the enzyme assay combination was not destroyed and produced a dark-blue color complex in the presence of inhibitors from the extracts, however no colored complex was produced in the absence of the inhibitor, showing that starch was completely hydrolyzed by -amylase. The extract's percentage inhibition was compared to that of acarbose, a reference medication. The IC50 of acarbose was established as a positive control. The percentage of -amylase inhibition was estimated using the formula below:

Percent inhibition = [(Abs of blank – Abs of sample) / Abs of blank] × 100

The absorbance of the blank is Abs of blank, whereas the absorbance of the sample is Abs of sample.

α-glucosidase inhibition Activity:

The inhibition of α-glucosidase activity was determined using the modified method of Shibano et al. (2008). In a 96-well plate, reaction mixture containing 20 μl of plant extract at varying concentrations (10, 20, 30, 40, 50, 60, 70 and 80 μg/ml) was make up with 1ml phosphate buffer (100 mm, pH = 6. 8), and 20 μl of 5 mm *p*-nitrophenyl α-D-glucopyranoside. After preincubating at 37°C for 5 min, 10 μl α-glucosidase (1.0 unit/ml) was added and incubated at 37°C for 15 min. The reaction was terminated by the addition of 50 μl Na_2CO_3 (0.1 M). The absorbance of the released p-nitro phenol was measured at 405 nm using Multi plate Reader (iMark™ Microplate Absorbance Reader). Acarbose at various concentrations (0.1–0.5 mg/ml) was included as a standard. Without test substance was set up in parallel as a control and each experiment was performed in triplicates. The results were expressed as percentage inhibition, which was calculated using the formula,

Inhibitory activity (%) = (1 – Sample Absorbance / Control Absorbance) ×100

Statistical analysis:

All of the tests were carried out in duplicate. The data is presented as a mean with standard error (SEM). One-way analysis of variance (ANOVA) was used to compare the differences between the extracts, followed by Duncan's multiple range test. The SAS programme version 9 was used to do the calculations.

Results and Discussion:

α-Amylase inhibitory activity:

As evidenced by the IC50 values, selected plant extracts considerably reduced -amylase inhibitory activity in the current investigation (Figure-1). Table-1 summarises the results, which were estimated as percentage inhibition (under experimental circumstances). At a concentration of 10 to 80 g/ml, *Acalypha fruticosa, Alangium salvifolium, Eryrthroxylum monogynum, Amarthus virudis*, and *Phyllanthus madraspetensis* showed 15.66 0.22 to 37.34 0.27, respectively, *Alangium salvifolium* showed 10.15 0.18 to 36.31 0.26, *Eryrthroxylum monogynum* showed.

Two extracts, *Eryrthroxylum monogynum* and *Phyllanthus madraspetensis*, showed considerable -amylase inhibitory action among the five plant species studied (*Acalypha fruticosa, Alangium salvifolium, Eryrthroxylum monogynum, Amarthus virudis,* and *Phyllanthus madraspetensis*). The inhibitory activity of these plants was also found to be concentration dependent. These plant extracts' dose-response curves were compared to those of acarbose as a control (Fig-1).

Pancreatic -amylase (E.C. 3.2.1.1) is a major digestive enzyme that catalyses the first stage in the hydrolysis of starch into a combination of smaller oligosaccharides that include maltose, maltotriose, and a number of -(l-6) and -(1-4) oligoglucans. These are subsequently digested by -glucosidases into glucose, which enters the bloodstream after absorption. This dietary starch degrades quickly, resulting in heightened post-prandial hyperglycemia (PPHG). The activity of HPA (human pancreatic -amylase) in the small intestine has been linked to an increase in post-prandial glucose levels, making its management an important part of T2DM treatment (7). As a result, slowing starch digestion by inhibiting enzymes like -amylase is important for diabetes management. Inhibitors of pancreatic -amylase slow carbohydrate digestion, lowering post-prandial serum glucose levels and reducing the rate of glucose absorption (8). Acarbose, for example, is an inhibitor of α-glucosidase and -amylase. Many of these synthetic hypoglycaemic medicines, however, have drawbacks, are non-specific, have substantial adverse effects, and do not improve diabetes problems. Bloating, abdominal discomfort, diarrhoea, and flatulence are the most common gastrointestinal adverse effects of these inhibitors (9). When compared to synthetic hypoglycemic drugs, herbal remedies are becoming more popular in the treatment of diabetes since they have less adverse effects and are less expensive (10).

α-Glucosidase inhibitory activity

Figure-2 shows the outcome of the inhibitory action of -glucosidase. One plant extract, Phyllanthus madraspetensis, showed considerable α-glucosidase inhibitory action among the five plant extracts studied (*Acalypha fruticosa*, *Alangium salvifolium*, *Eryrthroxylum monogynum*, *Amarthus virudis*, and *Phyllanthus madraspetensis*). The inhibitory activity of these plants was also found to be concentration dependent. These plant extracts' dose-response curves were compared to those of acarbose as a control (Figure-2). The dosage response curve was used to calculate the IC50 values. The IC50 values of several samples were determined using a wide range of concentrations of plant extracts that serve as inhibitors.

Acalypha fruticosa extract showed 29.87±0.18 to 68.58±0.47 percentage, *Alangium salvifolium* showed 47.57±0.42 to 66.66±0.48 percentage, *Eryrthroxylum monogynum* showed 36.33±0.28 to 56.95±0.44 percentage, *Amarthus virudis* showed 12.17±0.18 to 34.61±0.27 percentage of, and Acarbose was utilised as a standard reference medication, and it had inhibitory action against **α**-glucosidase with an IC50 value of 10 µg/ml and inhibitory activity against amylase with an IC50 value of 25.14 µg/ml.

Intestinal **α**-glucosidase is a crucial enzyme in carbohydrate digestion that has been identified as a therapeutic target for postprandial hyperglycemia control. Mammalian -glucosidases crude extract from rat intestinal mucosa, on the other hand, comprises a mixture of sucrose, maltase, isomaltase, and glucoamylase enzyme activities. The chromogenic substrate, pNPG, is a generic and non-specific substrate that reacts with all of the enzymes in the mixture, while the inhibitory reaction could be more selective and affect only a subset of them. As a result, the rate of inhibitory action is underestimated. So, when evaluating inhibitory activity of **α**-glucosidase, it is more relevant and interesting to use a specific substrate for each one, such as maltose, saccharide, or isomaltose, for greater precision and, on the other hand, to be closer to physiological conditions in the small intestine, where disaccharides are the major carbohydrate (11).

Table 1. Alpha amylase inhibitory activity of different concentrations of plant extracts, and acarbose.

Con (µg/ml)	*Acalypha fruticosa*	*Alangium salvifolium*	*Eryrthroxylum monogynum*	*Amaranthus viridis*	*Phyllanthus madraspetensis*	Standard Acarbose
10	15.66 ± 0.22	10.15 ± 0.18	51.23 ± 0.32	18.93 ± 0.18	18.19 ± 0.18	46.65 ± 0.52
20	18.76 ± 0. 20	11.70 ± 0.09	52.66 ± 0.28	21.96 ± 0.16	24.43 ± 0.22	49.86 ± 0.92
30	23.58 ± 0.18	12.73 ± 0.15	54.87 ± 0.26	24.26 ± 0.14	34.83 ± 0.28	52.54 ± 1.12
40	27.71 ± 0.14	19.44 ± 0.08	57.52 ± 0.34	25.30 ± 0.18	57.36 ± 0.30	55.40 ± 1.00
50	28.57 ± 0.17	23.75 ± 0.15	59.17 ± 0.36	27.71 ± 0.22	62.56 ± 0.42	57.77 ± 0.74
60	30.29 ± 0.18	24.95 ± 0.24	60.16 ± 0.40	29.50 ± 0.23	63.77 ± 0.38	58.25 ± 0.62
70	34.93 ± 0.17	28.74 ± 0.22	68.76 ± 0.38	33.04 ± 0.37	64.81 ± 0.33	60.40 ± 0.98
80	37.34 ± 0.27	36.31 ± 0.26	72.89 ± 0.44	44.47 ± 0.65	65.16 ± 0.47	62.45 ± 1.24

Acarbose was used as the positive control to ensure that the results were reliable. Values are expressed as mean triplicate determinations ± SEM; (n=3)

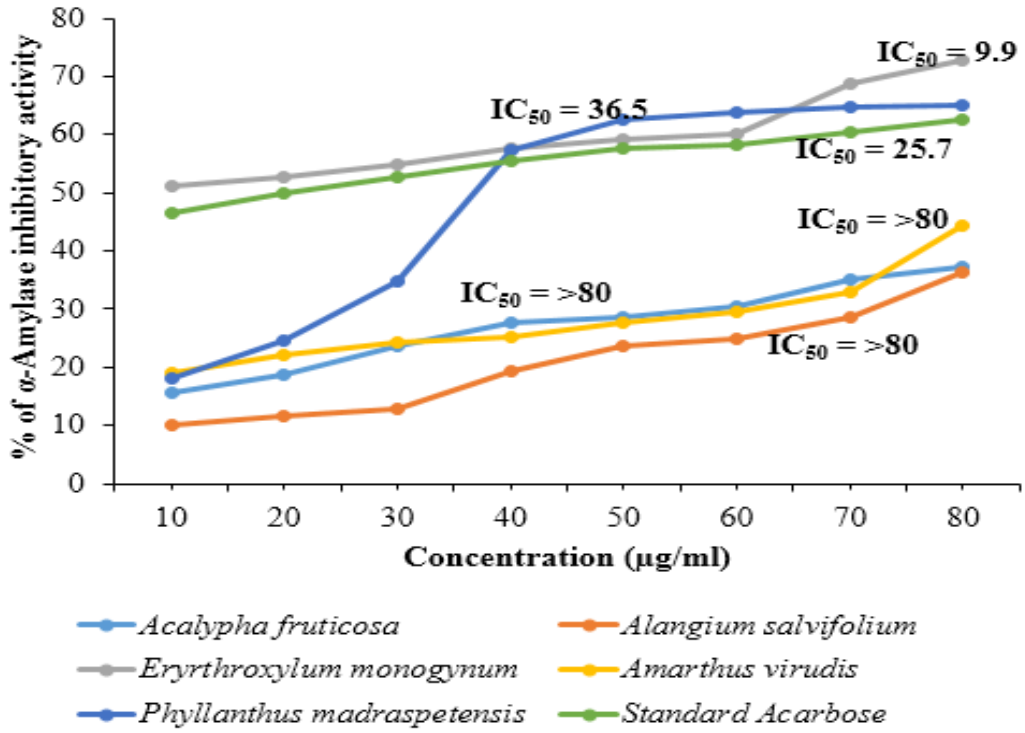


Figure-1. *In vitro* α-amylase inhibitory activity of selected medicinal plant extracts Acarbose is taken as standard inhibitor.

The plant extracts were found to have varying inhibitory effects on -amylase (Table 1) and **α**-glucosidase (Table 2). The methanol extract of *C. inophyllum* (IC50 = 75.10 µg/ml) had the strongest -amylase inhibitory activities, followed by *L. Frutescens* (IC50 = 50.21 µg/ml), *A. tennela* (IC50 = 35.25 g/ml), and *P. madraspetensis* (IC50 = 30.14 µg/ml), and the strongest -glucosidase

Acarbose-like medicines, which inhibit -glucosidase in the small intestine epithelium, have been shown to reduce post-prandial hyperglycemia and improve poor glucose metabolism in NIDMM patients without increasing insulin secretion (12). These drugs are most beneficial to those who have recently been diagnosed with T2DM and have blood glucose levels that are only slightly above the dangerous limit for diabetes. They're also helpful for patients who take sulfonylureas or metformin and need to take another medicine to keep their blood glucose levels in check. As a result, using a plant-based α-glucosidase inhibitor to slow and delay carbohydrate absorption could be a promising treatment method for T2DM control (13). Nonetheless, by lowering postprandial hyperglycemia, the extract appears to be promise in the treatment of T2DM. Acarbose anti-diabetic efficacy has been linked to gastrointestinal adverse effects induced by excessive inhibition of pancreatic -amylase, which results in aberrant bacterial fermentation of undigested carbohydrates in the large intestine. Any bioactive component with lesser inhibitory activity against -amylase and stronger inhibitory activity against α-glucosidase may be a viable therapeutic agent for the treatment of postprandial hyperglycemia with fewer adverse effects than acarbose, according to the evidence (13).

Table 2. Alpha Glucosidase inhibitory activity of different concentrations of five plant extracts, and acarbose

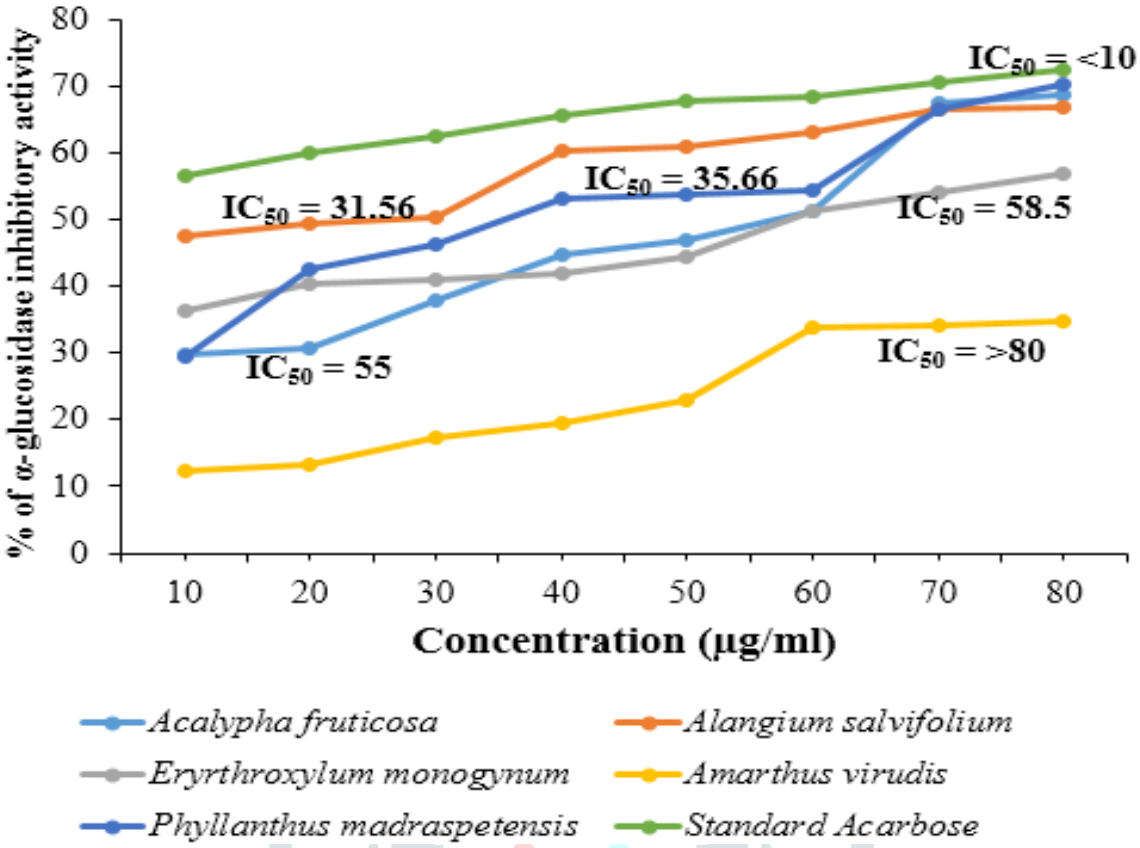
Con (µg/ml)	*Acalypha fruticosa*	*Alangium salvifolium*	*Eryrthroxylum monogynum*	*Amarthus virudis*	*Phyllanthus madraspetensis*	Standard Acarbose
10	29.87 ± 0.18	47.57 ± 0.42	36.33 ± 0.28	12.17 ± 0.18	29.46 ± 0.28	56.65 ± 0.42
20	30.64 ± 0.32	49.35 ± 0.29	40.46 ± 0.32	13.13 ± 0.09	42.63 ± 0.34	59.86 ± 0.48
30	37.94 ± 0.28	50.38 ± 0.41	40.94 ± 0.38	17.43 ± 0.15	46.16 ± 0.38	62.54 ± 0.54
40	44.61 ± 0.38	60.38 ± 0.54	41.79 ± 0.29	19.35 ± 0.20	53.14 ± 0.30	65.40 ± 0.58
50	46.92 ± 0.41	60.89 ± 0.48	44.22 ± 0.38	22.94 ± 0.24	53.73 ± 0.42	67.77 ± 0.61
60	51.15 ± 0.36	62.94 ± 0.37	51.23 ± 0.42	33.84 ± 0.30	54.32 ± 0.38	68.25 ± 0.57
70	67.30 ± 0.45	66.53 ± 0.45	54.03 ± 0.37	33.97 ± 0.28	66.55 ± 0.54	70.40 ± 0.48
80	68.58 ± 0.47	66.66 ± 0.48	56.95 ± 0.44	34.61 ± 0.27	70.31 ± 0.60	72.45 ± 0.64

Acarbose was used as the positive control to ensure that the results were reliable. Values are expressed as mean triplicate determinations ± SEM; (n=3)

Numerous studies have shown that the activities of -amylase and α-glucosidase have a major impact on blood glucose levels, and that inhibiting them could significantly reduce the postprandial rise in blood glucose (14). Several inhibitors of -amylase and α-glucosidase have been discovered from medicinal plants to serve as an alternative to existing synthetic medications with higher potency and fewer side effects (14).

Similar to our findings, Stephen et al. (2015) investigated the distribution of phenolic contents, anti-diabetic potentials, anti-hypertensive properties, and anti-oxidative effects of *Annona muricata* (*A. muricata*) fruit parts *in vitro* and discovered that *A. muricata* extracts inhibited α-glucosidase more than -amylase (15). Natural α-glucosidase inhibitors from plants have been demonstrated to exhibit substantial inhibitory activity against α-glucosidase in a small number of trials, suggesting that they could be employed as an effective medication for the management of AD and T2DM with few adverse effects (17). The findings of this study are promising, indicating that alpha-amylase inhibition may provide a safe approach or complement treatment plan for diabetes control. As a result, their generated products could be an important source of nutrition and therapy. Purification of the specific active ingredients is also required, which will aid in the creation of new pharmaceuticals for the treatment of diabetes mellitus.

Figure-2. *In vitro* α-glucosidase inhibitory activity of selected medicinal plant extracts. Acarbose is taken as standard inhibitor.



Conclusion:

When compared to the reference drug, *Eryrthroxylum monogynum* and *Phyllanthus madraspetensis* plant methanol extracts were found to be more potent against -amylase inhibitory activity and *Acalypha fruticosa* and *Phyllanthus madraspetensis* plant methanol extracts were found to be more potent against α-glucosidase inhibitory activity. All of the plants employed in this study are native to India and are well-known for their therapeutic characteristics among herbal pharmacologists. This study backs up their anti-diabetic claims with scientific facts. These plants are quite inexpensive for the average person and can simply be included in their daily diets. They can be studied further in order to develop anti-diabetic medications with fewer negative effects.

Figure-3. Plant Photos:

1. Acalypha fruticosa

2. Alangium salvifolium

2. Eryrthroxylum monogynum

4. Amaranthus viridis

5. Phyllanthus madraspetensis

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